

Claims

1. A crystal structure of the pRb/E2F₍₄₀₉₋₄₂₆₎ complex, characterised by the atomic co-ordinates of Annex 1.

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2. A crystal structure as claimed in claim 1, wherein the interactions between E2F₍₄₀₉₋₄₂₆₎ and pRb comprise one or more of the following interactions:

E2F ₍₄₀₉₋₄₂₆₎ residue	pRb residue
Leu ₄₀₉	Lys ₅₄₈
Tyr ₄₁₁	Glu ₅₅₁
Tyr ₄₁₁	Ile ₅₃₂
Tyr ₄₁₁	Glu ₅₅₄
His ₄₁₂	Arg ₆₅₆
His ₄₁₂	Lys ₆₅₃
Gly ₄₁₄	Glu ₅₃₃
Gly ₄₁₄	Lys ₆₅₂
Leu ₄₁₅	Leu ₆₄₉
Leu ₄₁₅	Glu ₅₅₃
Leu ₄₁₅	Lys ₅₃₇
Glu ₄₁₇	Lys ₅₃₇
Gly ₄₁₈	Arg ₄₆₇
Glu ₄₁₉	Thr ₆₄₅
Arg ₄₂₂	Glu ₄₆₄
Asp ₄₂₃	Arg ₄₆₇
Leu ₄₂₄	Lys ₅₃₀
Phe ₄₂₅	Phe ₄₈₂
Phe ₄₂₅	Lys ₄₇₅

3. A method to identify an agent which modulates the interaction between pRb and E2F₍₄₀₉₋₄₂₆₎, the method comprising:
- 5 a) combining together pRb, E2F₍₄₀₉₋₄₂₆₎ and an agent, under conditions in which pRb and E2F₍₄₀₉₋₄₂₆₎ form a complex;
- b) growing a crystal structure of any pRb/ E2F₍₄₀₉₋₄₂₆₎ complex; and
- 10 c) analysing the crystal to determine whether the agent is an agent which modulates the interaction between pRb and E2F₍₄₀₉₋₄₂₆₎.
4. A method, as claimed in claim 3, wherein the combining of the components is pRb with the agent and then E2F₍₄₀₉₋₄₂₆₎.
- 15 5. A method as claimed in claim 3, wherein the combining of the components is E2F₍₄₀₉₋₄₂₆₎ with the agent and then pRb.
6. A method as claimed in claim 3, wherein the combining of the components is pRb with E2F₍₄₀₉₋₄₂₆₎ and then the agent.
- 20 7. A method as claimed in any one of claims 3 to 6, wherein step c) comprises comparing the crystal structure to the crystal structure of claim 1
8. A method as claimed in any one of claims 3 to 7, wherein the agent is selected
- 25 using the three dimensional atomic co-ordinates of Annex 1.
9. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex, comprising selecting an agent using the three-dimensional atomic coordinates of Annex 1.

10. A method as claimed in claim 9, wherein said selection is performed in conjunction with computer modeling.
11. A method as claimed in claim 9 or 10, wherein the method further comprises the steps of:
- 5 a) contacting the selected agent with pRb and E2F₍₄₀₉₋₄₂₆₎ under conditions in which pRb and E2F₍₄₀₉₋₄₂₆₎ can form a complex; and
- b) measuring the binding affinity of pRb to E2F₍₄₀₉₋₄₂₆₎ in the presence of the agent and comparing the binding affinity to that of pRb to E2F₍₄₀₉₋₄₂₆₎ when in the
- 10 absence of the agent, wherein an agent modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex when there is a change in the binding affinity of pRb to E2F₍₄₀₉₋₄₂₆₎ when in the presence of the agent.
12. A method as claimed in claim 11, wherein the method further comprising:
- 15 a) growing a supplementary crystal from a solution containing pRb and E2F₍₄₀₉₋₄₂₆₎ and the selected agent where said agent changes the binding affinity of the pRb/E2F₍₄₀₉₋₄₂₆₎ complex under conditions in which pRb and E2F₍₄₀₉₋₄₂₆₎ can form a complex;
- b) determining the three-dimensional atomic coordinates of the supplementary
- 20 crystal by X-ray diffraction using molecular replacement analysis;
- c) comparing the three dimensional coordinates with those for the crystal structure as claimed in claim 1; and
- d) selecting a second generation agent using the three-dimensional atomic coordinates determined for the supplementary crystal.
- 25 13. A method as claimed in claim 12, wherein said selection is performed in conjunction with computer modeling.
14. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex,
- 30 comprising:

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- a) contacting a selected agent with pRb and E2F₍₄₀₉₋₄₂₆₎ under conditions in which pRb and E2F₍₄₀₉₋₄₂₆₎ can form a complex; and
 - b) measuring the binding affinity of pRb to E2F₍₄₀₉₋₄₂₆₎ in the presence of the agent and comparing the binding affinity to that of pRb to E2F₍₄₀₉₋₄₂₆₎ when in the absence of the agent, wherein an agent modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex when there is a change in the binding affinity of pRb to E2F₍₄₀₉₋₄₂₆₎ when in the presence of the agent.

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15. A method as claimed in claim 14, wherein the method further comprising:
- a) growing a supplementary crystal from a solution containing pRb and E2F₍₄₀₉₋₄₂₆₎ and the selected agent where said agent changes the binding affinity of the pRb/E2F₍₄₀₉₋₄₂₆₎ complex under conditions in which pRb and E2F₍₄₀₉₋₄₂₆₎ can form a complex;
 - b) determining the three-dimensional atomic coordinates of the supplementary crystal by X-ray diffraction using molecular replacement analysis;
 - c) comparing the three dimensional coordinates with those for the crystal structure claimed in claim 1; and
 - d) selecting a second generation agent using the three-dimensional atomic coordinates determined for the supplementary crystal.

16. A method as claimed in claim 15, wherein said selection is performed in conjunction with computer modeling.

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17. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex, comprising:
- a) selecting an agent;
 - b) co-crystalising pRb with the agent;
 - c) determining the three dimensional coordinates of the pRb-agent association by X-ray diffraction using molecular replacement analysis; and

- d) comparing the three dimensional coordinates with those of the crystal structure claimed in claim 1.

18. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex,
5 comprising:
a) selecting an agent;
b) crystalising pRb and soaking the agent into the crystal;
c) determining the three dimensional coordinates of the pRb-agent association by X-ray diffraction using molecular replacement analysis; and
10 d) comparing the three dimensional coordinates with those of the crystal structure claimed in claim 1.

19. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex,
comprising:
15 a) selecting an agent;
b) co-crystalising pRb, E2F₍₄₀₉₋₄₂₆₎ and the agent;
c) determining the three dimensional coordinates of the pRb-E2F-agent association by X-ray diffraction using molecular replacement analysis; and
d) comparing the three dimensional coordinates with those of the crystal structure
20 claimed in claim 1.

20. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex,
comprising:
a) selecting an agent;
25 b) co-crystalising pRb and E2F₍₄₀₉₋₄₂₆₎ and soaking the agent into the crystal;
c) determining the three dimensional coordinates of the pRb-E2F-agent association by X-ray diffraction using molecular replacement analysis; and
d) comparing the three dimensional coordinates with those of the crystal structure claimed in claim 1.

21. A method as claimed in any one of claims 17 to 20, wherein the agent is selected using the three dimensional atomic co-ordinates of Annex 1
22. A method as claimed in any one of claims 17 to 21, wherein the methods further
5 comprise selecting a second generation agent using the three dimensional atomic coordinates determined in step c).
23. A method as claimed in claim 22, wherein the second generation agent is selected using the three dimensional atomic coordinates of Annex 1.
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24. A method as claimed in claim 22 or 23, wherein the selection is performed in conjunction with computer modeling.
25. A method of identifying an agent as claimed in any one of claims 3 to 24, wherein
15 the selected agent and/or the second generation agent mimics a structural feature of E2F₍₄₀₉₋₄₂₆₎, when said E2F₍₄₀₉₋₄₂₆₎ is bound to pRb.
26. A method as claimed in claim 9 or 10, wherein method comprises the further steps of:
20 a) contacting the selected agent with a pRb/E2F₍₄₀₉₋₄₂₆₎ complex; and
b) determining whether the agent affects the stability of the complex.
27. A method as claimed in claim 26, wherein the determination is with fluorescence polarization.
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28. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex, comprising:
a) contacting a fluorescently tagged E2F₍₄₀₉₋₄₂₆₎ peptide (E2F-fluoropeptide) with pRb to allow pRb/E2F-fluoropeptide complex formation;
30 b) detecting the fluorescence polarization;

- c) adding a selected agent; and
- d) detecting the fluorescence polarization in the presence of the agent.

29. A method of identifying an agent that modulates a pRb/E2F₍₄₀₉₋₄₂₆₎ complex,
5 comprising;

- a) contacting a fluorescently tagged E2F₍₄₀₉₋₄₂₆₎ peptide (E2F-fluoropeptide) with pRb to allow pRb/E2F-fluoropeptide complex formation;
- b) detecting the fluorescence polarization;
- c) contacting a selected agent with pRb and E2F₍₄₀₉₋₄₂₆₎ peptide (E2F-fluoropeptide)
10 under conditions in which pRb and E2F-fluoropeptide can form a complex;
- d) detecting the fluorescence polarization; and
- e) comparing the fluorescence polarization detected in b) and d).

30. A method as claimed in claim 28 or 29, wherein the fluorescently tagged E2F
15 peptide is selected using the three dimensional atomic co-ordinates of Annex 1.

31. A method as claimed in any one of claims 28 to 30, wherein a decrease in
fluorescence polarization in the presence of the agent indicates that the agent
destabilises the complex.

20 32. A method as claimed in any one of claims 28 to 31, wherein the method
comprises the further step of adding untagged E2F₍₄₀₉₋₄₂₆₎ and detecting
fluorescence polarization.

25 33. A method as claimed in claim 32, wherein if fluorescence polarization decreases,
on addition of the untagged E2F₍₄₀₉₋₄₂₆₎, the agent does not stabilise the complex.

30 34. A method as claimed in claim 32 or 33, wherein if there is no substantial change in
fluorescence polarization, on addition of the untagged E2F₍₄₀₉₋₄₂₆₎, the agent
stabilises the complex.

35. A method as claimed in any one of claims 28 to 34, wherein the method further comprises:
- a) contacting a fluorescently tagged E7 peptide (E7-fluoropeptide) with pRb to allow pRb/E7-fluoropeptide complex formation;
 - b) detecting the fluorescence polarization;
 - c) adding an agent that modulates pRb/E2F₍₄₀₉₋₄₂₆₎ complex; and
 - d) detecting the fluorescence polarization in the presence of the agent.
36. A method as claimed in any one of claims 28 to 34, wherein the method further comprises:
- a) contacting a fluorescently tagged E7 peptide (E7-fluoropeptide) with pRb to allow pRb/E7-fluoropeptide complex formation;
 - b) detecting the fluorescence polarization;
 - c) contacting an agent that modulates pRb/E2F₍₄₀₉₋₄₂₆₎ complex with pRb and E7-fluoropeptide under conditions in which pRb and E7-fluoropeptide can form a complex;
 - d) detecting the fluorescence polarization; and
 - e) comparing the fluorescence polarization detected in b) and d).
37. A method as claimed in claim 35 or 36, wherein a decrease in fluorescence polarization indicates that the agent also inhibits E7 binding to pRb.
38. A method as claimed in any one of claims 11 to 16, wherein the binding affinity is measured by isothermal titration calorimetry.
39. A method as claimed in any one of claims 11 to 16, wherein the binding affinity is measured by Surface Plasmon Resonance (SPR).

40. An agent, that modulates the interaction between pRb and E2F₍₄₀₉₋₄₂₆₎, identified by a method as claimed in any one of claims 3 to 39.
- 5 41. An agent, as claimed in claim 40, for use as an apoptosis promoting factor in the prevention or treatment of proliferative diseases.
42. An agent as claimed in claim 40 or 41, wherein the agent is for use in preventing or treating cancer, which may be pancreatic cancer and related diseases.
- 10 43. The use of an agent, which modulates the formation of a pRb/E2F₍₄₀₉₋₄₂₆₎ complex, identified by a method as claimed in any one of claims 3 to 39, in the manufacture of a medicament for the prevention or treatment of proliferative diseases.
- 15 44. The use of an agent as claimed in claim 43, wherein the proliferative diseases are cancer, preferably pancreatic cancer and related diseases.
45. The use of the atomic co-ordinates of the crystal structure as claimed in claim 1 or 2, for identifying an agent that modulates the formation of a pRb/E2F₍₄₀₉₋₄₂₆₎ complex.
- 20 46. Computer readable media comprising a data storage material encoded with computer readable data, wherein said computer readable data comprises a set of atomic co-ordinates of the pRb/E2F₍₄₀₉₋₄₂₆₎ crystal structure of Annex 1 recorded thereon.